Supplement S 4. explanation of frequency response analysis results (FRA)

In a frequency response analysis, the dynamic behavior of a system is investigated by providing a harmonic oscillation as input signal, described by a sine wave with a variable frequency. Subsequently, the amplitude and the phase of the resulting sine wave are compared to that of the original sine wave. This frequency response analysis is often applied to a linear or linearized system and is derived analytically from the system's differential equations, but can also be derived from experimental data or simulations (Ang et al., 2011). Dopamine concentrations are released in a pulsatile manner with a frequency of around 1 s^{-1}, but also show more slowly fluctuating levels caused by transient activity. Therefore, a frequency response analysis with fluctuating dopamine concentrations as input is reflective of the relevant context of drug action for dopamine antagonists.

Here we show the intermediate steps that lead to the eventual calculation of gain in cAMP amplitude. Firstly, we show in the top row of three examples of different input frequencies, for which the different line colors all have the same overlaying sine wave characteristics and are not influenced by the drug-target k_{off}.

The second row of shows how the dopamine occupancy follows the dopamine concentrations (in a non-linear fashion) until the frequency gets too high, as visible for the highest frequency, and the amplitude of the dopamine occupancy fluctuation declines. The slight influence of the antagonist k_{off} on dopamine occupancy for the intermediate occupancy can be explained by the competitive binding of the antagonist and dopamine and the influence of the koff on antagonist binding, as shown in the third row of .

The third row of shows how all antagonists can be displaced by dopamine binding for the slowest frequency (hence the fluctuating occupancy), while for the intermediate frequency only the fast dissociating drug can be displaced fast enough to keep the original amplitude. Finally, for the highest frequency, none of the antagonists can be displaced fast enough and the fluctuation in dopamine concentrations and dopamine occupancy is not reflected in the antagonist-receptor occupancy.

The bottom row of shows how the differences in dopamine and antagonist occupancy are translated into cAMP concentrations in a frequency-dependent manner (note the increased gain for the intermediate frequency that is not reflected in the occupancy profiles).

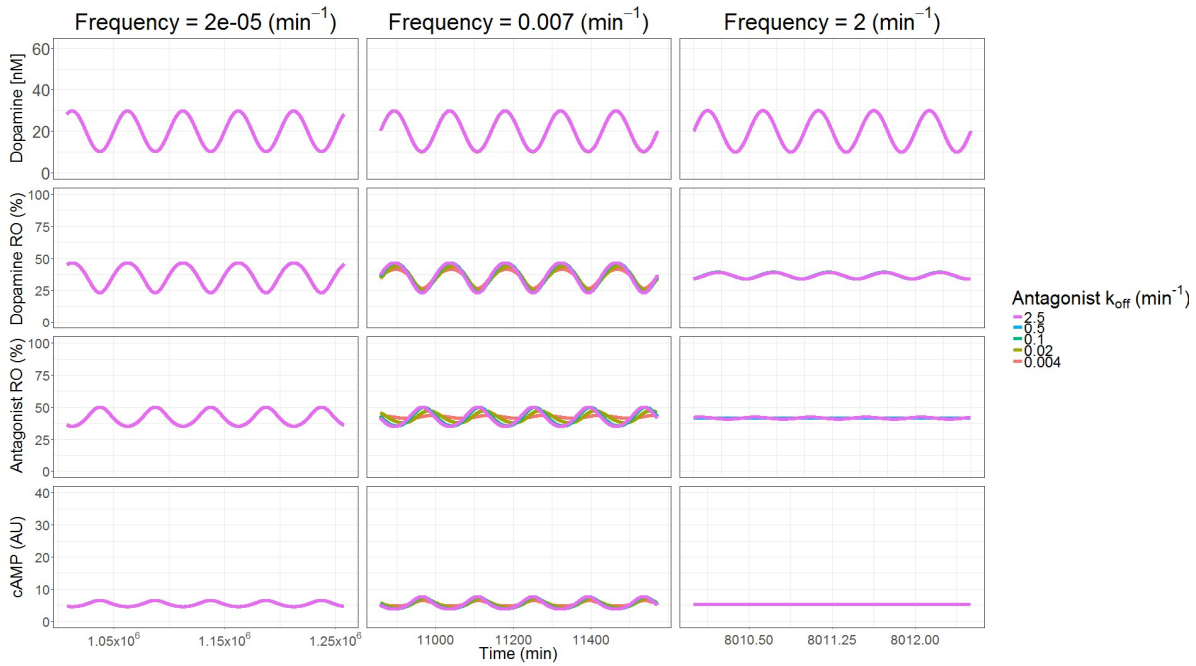

Figure S 4. Example of input frequencies for dopamine as used in the simulations (top panels) and the simulated responses (lower panels). The second row shows the dopamine receptor occupancy, the third row the antagonist receptor occupancy and the bottom row the cAMP response for each simulation with the fluctuating dopamine concentrations from the corresponding top row panels. The different line colors represent different simulations for which the dissociation rate constant of the antagonist-receptor complex is changed. The dopamine fluctuation frequencies are indicated above the panels and by the different time scales on the x-axis.